Quantikine® ELISA

Mouse/Rat SOST Immunoassay

Catalog Number MSST00

For the quantitative determination of mouse or rat SOST concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

SOST, also known as Sclerostin, is a Cerberus/DAN family member and is an important regulator of bone homesotasis (1). Cerberus/DAN proteins (Cerberus, DAN, Gremlin, PRDC, and Caronte) are secreted glycoproteins that function as BMP antagonists. While the overall sequence identity between members of the family is low, they share a cysteine-knot motif with conserved spacing of six cysteine residues. SOST is secreted as a monomer in contrast to many other cysteine-knot proteins which form disulfide-linked homodimers (2). Mature mouse SOST shares 89% and 95% amino acid sequence identity with human and rat SOST, respectively (3, 4). Inactivating mutations in the SOST gene can cause sclerosteosis and van Buchem disease which are bone dysplasia disorders characterized by progressive skeletal overgrowth (3-5). SOST is expressed by terminally differentiated cells embedded in mineralized matrix including osteocytes (6, 7), hypertrophic and prehypertrophic chondrocytes (7-9), and tooth cementocytes (8). SOST expression is induced by BMP-2, -4, and -6 (10, 11) and is inhibited by parathyroid hormone (PTH) (12, 13). Its downregulation in osteocytes by physical loading of bone contributes to the mechanical sensor function of osteocytes and the subsequent increase in bone growth (14).

SOST binds to BMP-2, -4, -5, -6, and -7 and inhibits the alkaline phosphatase (ALP) activity induced by these BMPs (2, 6, 7). It inhibits canonical Wnt signaling by binding to LRP-5 and LRP-6 and inhibiting their association with Frizzled receptors (15, 16). SOST also modulates the ability of BMPR-IA signaling to interfere with canonical Wnt signaling (17). These interactions underlie the ability of SOST to inhibit BMP- and Wnt-induced bone formation *in vivo* (17, 18). SOST reduces the proliferation of mesenchymal stem cells (MSC) and induces MSC apoptosis (7, 19). It inhibits the differentiation of preosteoblastic cells and bone mineralization by osteoblasts (6, 7). In knockout mice that lack SOST expression, osteocyte and osteoblast apoptosis is inhibited, osteoblast activity is enhanced, and the mice are resistant to mechanical unloading-induced bone loss (20). SOST knockout mice also exhibit increased bone mineral density, bone volume, and bone strength throughout the skeleton in trabecular and cortical bone (21). Mice treated with neutralizing anti-SOST antibodies likewise show increased bone formation and bone mineral density (22, 23). This treatment can reverse the bone loss and bone integrity decline that is otherwise seen in models of osteoporosis and chronic gut inflammation (22, 23).

The Quantikine Mouse/Rat SOST immunoassay is a 4.5 hour solid-phase ELISA designed to measure SOST in mouse or rat cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse SOST and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse SOST. Results obtained using natural mouse or rat SOST showed dose response curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural SOST.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat SOST has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any SOST present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat SOST is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of SOST bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat SOST Microplate	894130	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat SOST.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat SOST Conjugate	894131	12 mL of a polyclonal antibody specific for mouse/rat SOST conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat SOST Standard	894132	5000 pg of recombinant mouse SOST in a buffered protein base with preservatives; lyophilized.	
Mouse/Rat SOST Control	894133	Recombinant mouse SOST in a buffered protein base with preservatives; lyophilized. The concentration range of SOST after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-12	895214	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

Rat serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-12.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

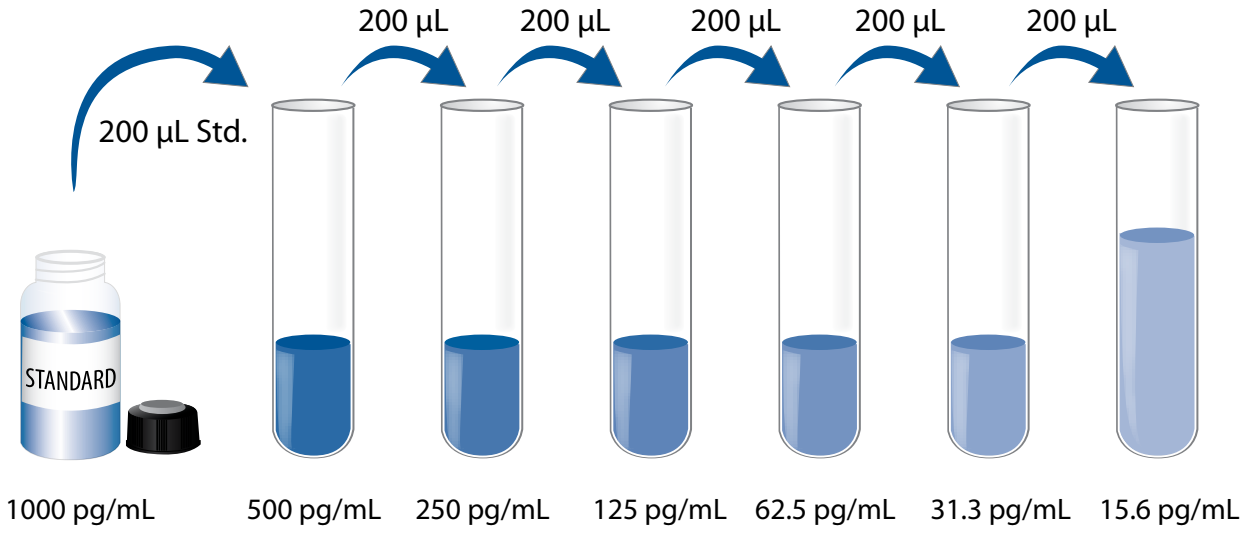
Mouse/Rat SOST Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse/Rat SOST Standard - Reconstitute the Mouse/Rat SOST Standard with 5.0 mL of Calibrator Diluent RD6-12. This reconstitution produces a stock solution of 1000 pg/mL. Allow the stock solution to sit for a minimum of 15 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD6-12 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse/Rat SOST Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD6-12 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, Control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Gently tap the plate to ensure thorough mixing. Cover with the adhesive strip provided. Incubate for **3 hours** at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse/Rat SOST Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light.
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Rat serum and plasma samples require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

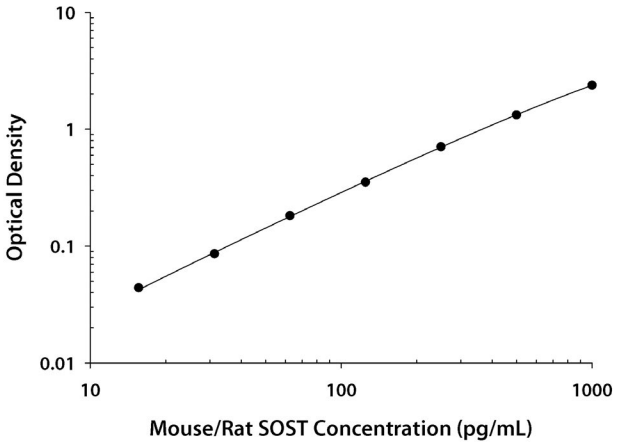
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse/rat SOST concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.025	0.024	—
15.6	0.067 0.068	0.068	0.044
31.3	0.106 0.113	0.110	0.086
62.5	0.205 0.207	0.206	0.182
125	0.374 0.378	0.376	0.352
250	0.710 0.754	0.732	0.708
500	1.320 1.381	1.351	1.327
1000	2.344 2.458	2.401	2.377

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	57.6	108	345	54.4	101	347
Standard deviation	4.15	6.00	18.7	4.35	6.23	19.4
CV (%)	7.2	5.6	5.4	8.0	6.2	5.6

RECOVERY

The recovery of SOST spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=4)	101	92-112%
Mouse serum (n=4)	95	86-113%
Mouse EDTA plasma (n=4)	98	82-113%
Mouse heparin plasma (n=4)	102	88-115%
Rat serum* (n=4)	99	85-116%
Rat EDTA plasma* (n=2)	107	101-118%
Rat heparin plasma* (n=2)	96	82-111%

*Samples were diluted prior to assay as described in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of SOST in each matrix were diluted with Calibrator Diluent and assayed.

		Cell culture media (n=6)	Mouse Serum (n=4)	Mouse EDTA plasma (n=4)	Mouse Heparin plasma (n=4)	Rat Serum* (n=4)	Rat EDTA plasma* (n=4)	Rat Heparin plasma* (n=4)
1:2	Average % of Expected	102	107	104	101	111	111	109
	Range (%)	100-106	105-109	100-108	98-105	110-112	105-115	106-115
1:4	Average % of Expected	106	109	104	103	114	112	109
	Range (%)	98-113	107-111	101-108	99-109	110-117	101-117	104-113
1:8	Average % of Expected	102	109	104	105	113	110	105
	Range (%)	95-112	105-113	98-109	98-116	107-116	102-119	96-116

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Sixty-two assays were evaluated and the minimum detectable dose (MDD) of SOST ranged from 0.422-4.17 pg/mL. The mean MDD was 1.63 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse SOST produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Mouse and rat samples were evaluated for the presence of SOST in this assay.

Mouse	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	184	142-248	36.3
EDTA plasma (n=10)	239	203-292	27.2
Heparin plasma (n=10)	193	132-239	36.8

Rat	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	316	232-465	92.0
EDTA plasma (n=10)	214	146-255	30.8
Heparin plasma (n=10)	209	165-252	26.0

Cell Culture Supernates:

Organs from mice and rats were removed, rinsed in PBS, and kept on ice. Organs were then homogenized using a tissue homogenizer and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 1 day. Aliquots of the cell culture supernates were removed and assayed for levels of natural SOST.

Tissue Type	(pg/mL)
Mouse kidney	28.4
Rat kidney	30.1

D3 mouse embryonic stem cells were cultured in DMEM supplemented with 2% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate, and incubated for 7 days. An aliquot of the cell culture supernate was removed, assayed for levels of mouse SOST, and measured 52.2 pg/mL.

Mouse bone marrow mast cells isolated from three 7-8 week old NSA mice were cultured in 100 mL RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 µM β-mercaptoethanol, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 7 days. The cells were either unstimulated or stimulated with 25 ng/mL recombinant mouse SCF (R&D Systems, Catalog # 455-MC) for 7 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse SOST.

Condition	Detectable Level (pg/mL)
Unstimulated	ND
Stimulated	84.5

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat SOST.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse/rat SOST control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

BAMBI/NMA
BMP-3
BMP-4
BMP-5
BMP-6
BMP-7
BMP-8
BMP-9
BMP-10
BMP-15
BMPR-IA
BMPR-IB
Cerberus 1
Chordin
Cripto
DAN
Follistatin
Gremlin
LRP-5
LRP-6
Noggin
TSG

Recombinant human:

BMP-2
BMP-4
BMP-5
Noggin

Recombinant human SOST shows 0.22% cross-reactivity in this assay.

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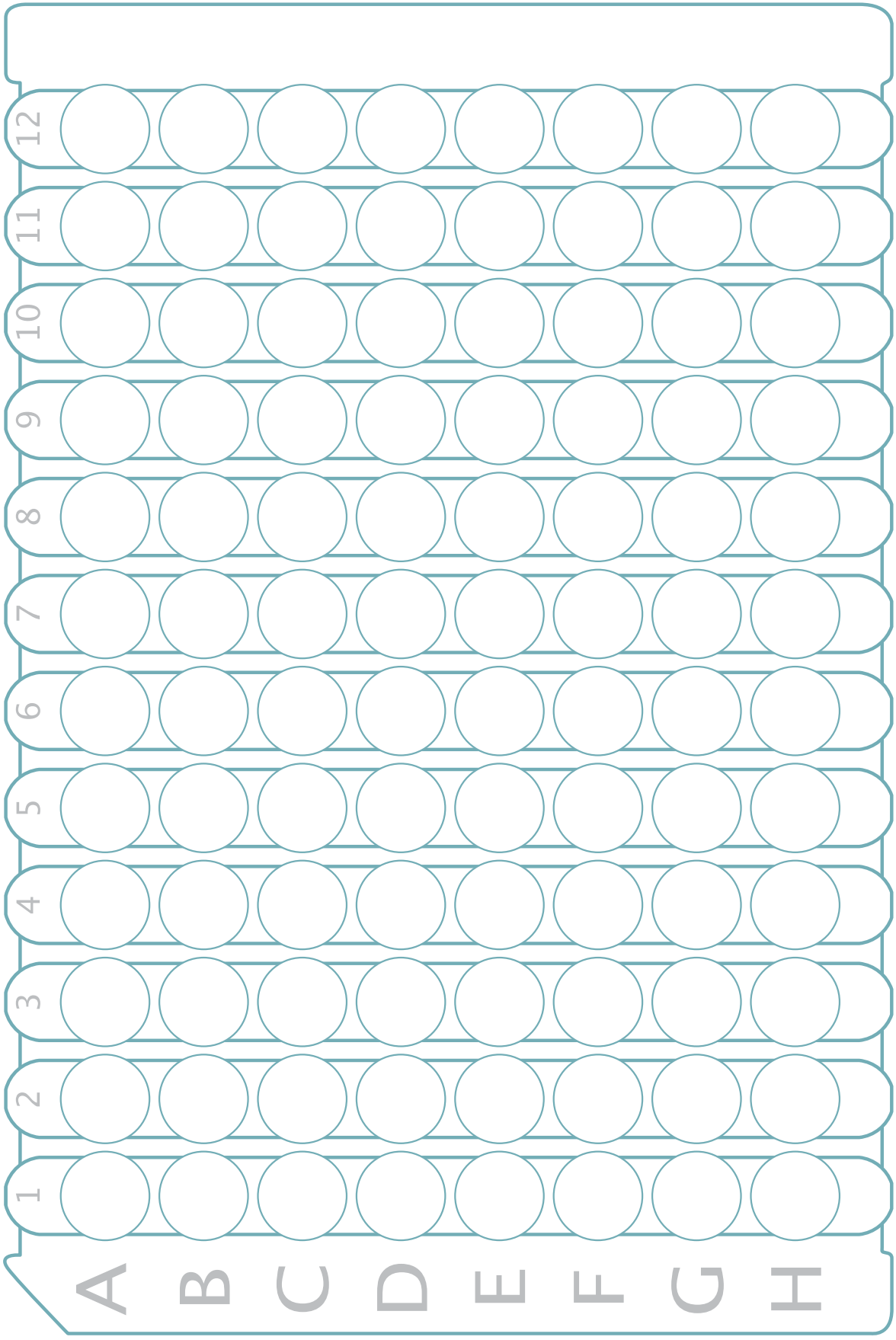
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES